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Published in:

International Journal of Infectious Diseases

DOI (link to publication from Publisher):

[10.1016/j.ijid.2018.01.025](https://doi.org/10.1016/j.ijid.2018.01.025)

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Publication date:

2018

Document Version

Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):

Lützen, L., Olesen, B., Voldstedlund, M., Christensen, J. J., Moser, C., Knudsen, J. D., Fuursted, K., Hartmeyer, G. N., Chen, M., Søndergaard, T. S., Rosenvinge, F. S., Dzajic, E., Schønheyder, H. C., & Nørskov-Lauritsen, N. (2018). Incidence of HACEK bacteraemia in Denmark: A 6-year population-based study. *International Journal of Infectious Diseases*, 68, 83-87. <https://doi.org/10.1016/j.ijid.2018.01.025>

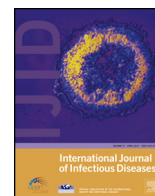
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Incidence of HACEK bacteraemia in Denmark: A 6-year population-based study

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ARTICLE INFO

Article history:

Received 17 October 2017

Received in revised form 22 January 2018

Accepted 24 January 2018

Keywords:

Epidemiology

Incidence

Age

Sex

Haemophilus

Aggregatibacter

ABSTRACT

Objectives: Bacteria with common microbiological and clinical characteristics are often recognized as a particular group. The acronym HACEK stands for five fastidious genera associated with infective endocarditis (*Haemophilus*, *Aggregatibacter*, *Cardiobacterium*, *Eikenella*, and *Kingella*). Data on the epidemiology of HACEK are sparse. This article reports a 6-year nationwide study of HACEK bacteraemia in Denmark.

Methods: Cases of HACEK bacteraemia occurring during the years 2010–2015 were retrieved from the national Danish microbiology database, covering an average surveillance population of 5.6 million per year.

Results: A total of 147 cases of HACEK bacteraemia were identified, corresponding to an annual incidence of 0.44 per 100 000 population. The annual incidence for males was 0.56 per 100 000 and for females was 0.31 per 100 000. The median age was 56 years (range 0–97 years), with variation among the genera. One hundred and forty-three isolates were identified to the species level and six to the genus level: *Haemophilus* spp, *n* = 55; *Aggregatibacter* spp, *n* = 37; *Cardiobacterium* spp, *n* = 9; *Eikenella* *corrodens* *n* = 21; and *Kingella* spp, *n* = 27.

Conclusions: This is the first study on the incidence of HACEK bacteraemia in a large surveillance population and may inspire further studies on the HACEK group. *Haemophilus* spp other than *Haemophilus influenzae* accounted for most cases of HACEK bacteraemia in Denmark, with *Aggregatibacter* spp in second place.

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Introduction

The HACEK group of bacteria comprises Gram-negative, fastidious rods that preferentially colonize the oropharynx and upper respiratory tract of humans. The species in this group are recognized as causes of infective endocarditis (IE) and are responsible for 1.4–3% of such cases (Ashrafi et al., 2012; Chambers et al., 2013; Das et al., 1997; Marks et al., 2015). A recent retrospective study from New Zealand with 87 cases of HACEK bacteraemia confirmed a strong association with IE (Yew et al., 2014). The overall prevalence proportion of IE was 60%, varying from 0% (*Eikenella corrodens*) to 100% (*Aggregatibacter actinomycetemcomitans*) (Yew et al., 2014). However, the clinical spectrum is much broader and includes pyogenic infections of the central nervous system, bones, joints, and soft tissue and localized infections in contact with mucosal surfaces (El Houmami et al., 2016; Goldberg and Katz, 2006; Nørskov-Lauritsen, 2014; Revest et al., 2016; Wang et al., 2010; Yagupsky, 2015; Yew et al., 2014).

The HACEK group was originally described to encompass *Haemophilus* species, *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, and *Kingella kingae* (Geraci and Wilson, 1982). According to recent taxonomic rearrangements, a new genus *Aggregatibacter* now accommodates bacteria formerly classified as *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus*, and *Haemophilus segnis* (Nørskov-Lauritsen and Kilian, 2006). Moreover, a new species, *Cardiobacterium valvarum*, associated with IE has been described (Bonavent et al., 2016; Chen et al., 2011; Han et al., 2004). Within the genus *Kingella*, *K. kingae* is the main species responsible for invasive diseases, although cases of *Kingella denitrificans* IE have been reported (Swann and Holmes, 1984). *Haemophilus influenzae* is rarely cultured from cases of IE and is often excluded from the HACEK group (Baron et al., 2005; Brouqui and Raoult, 2001; Petti et al., 2006). Thus, the HACEK acronym now denotes *Haemophilus* species other than *H. influenzae*, *Aggregatibacter* species, *Cardiobacterium* species, *E. corrodens*, and *Kingella* species.

While the previous HACEK literature has focused mainly on IE, knowledge of the incidence of HACEK bacteraemia and patient demography is still limited. The aim of this study was to describe the incidence of HACEK bacteraemia in Denmark for the years 2010–2015 regarding the distribution of the bacterial species in relation to patient sex and age.

Methods

The HACEK acronym was defined as above.

All Danish residents have unrestricted access to tax-paid health care from general practitioners and hospitals. Dental care is covered only partially by the tax system; children have full coverage, but adult care is based on user charges with the exception of vulnerable groups.

Health services are organized into five regions, with one to four departments of clinical microbiology per region. In 2010 the total number was 13, and this reduced to 11 during the study period. The BacT/Alert (bioMérieux, France) and the BACTEC (Becton Dickinson, USA) blood culture systems were used, with the former being the system most often used (information obtained from local co-authors). Electronic reporting of microbiology laboratory results became compulsory in Denmark on January 1, 2010, and

a copy of every report is submitted to the Danish Microbiology Database (MiBa) (Voldstedlund et al., 2014). Based on this routine, MiBa contains complete nationwide microbiology data. The MiBa database was searched and all patients with positive blood culture (s) with a HACEK species during the period January 1, 2010 through December 31, 2015 were identified.

An incident case was defined by the first positive blood culture with a HACEK species within this period. A case comprised one or more positive blood cultures with identical isolates within 30 days. Subsequent cultures were defined as a recurrent case. The bacterial identification reported by the local department of clinical microbiology was used without further confirmation. Most identifications were obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). A minority of early reports were based on phenotypic testing according to national (DSKM, 2011, 2012) and international guidelines (Versalovic et al., 2011).

All blood cultures were taken from patients admitted to hospital.

The surveillance population data (residents at the beginning of the first quarter of the year) were obtained from Statistics Denmark (<http://www.statistikbanken.dk>). Incidence rates were determined for the following age groups: infants 0–1 years, children and adolescents 2–19 years, adults 20–59 years, and older population 60+ years. Differences between the sexes were evaluated by calculation of the incidence rate ratio (Stata 10; Stata Corp., College Station, TX, USA).

The study was conducted in accordance with regional guidelines for the use of clinical and laboratory data and was approved by the Danish Data Protection Agency (record number 2012-58-0004) and the National Board of Health (record number 3-3013-1170/1). According to Danish law, register-based studies do not require approval from a committee on health research ethics.

Results

During the 6-year study period, the average surveillance population was 5.6 million, amounting to 33.6 million person-years at risk. A total of 147 incident cases of HACEK bacteraemia were retrieved, corresponding to an annual incidence of 0.44 per 100 000 population. The incidence varied little between the five Danish health regions (0.39–0.49 per 100 000 person-years). The annual numbers of cases were 17, 24, 29, 27, 30, and 20, with no definite time trend (range of annual incidences 0.30–0.54 per 100 000 population).

Ninety-six percent of the isolates were identified to the species level, while 4% were identified only to the genus level. The distribution of genera and species is shown in Table 1. *Haemophilus* was the most frequently identified genus, followed by *Aggregatibacter* and *Kingella* (incidence 0.16, 0.11, and 0.08 per 100 000 person-years, respectively). The 147 incident cases had 149 HACEK isolates, as two patients had a double infection, one with *Haemophilus parainfluenzae* and *Aggregatibacter segnis* and the other with *Aggregatibacter aphrophilus* and *E. corrodens*. Two patients had recurrent episodes of bacteraemia with the same species (*C. hominis* after 39 days and again after nearly 2 years, and *E. corrodens* after 44 days, respectively).

As compared with females, males had a higher incidence of HACEK bacteraemia (0.56 vs. 0.31 per 100 000 person-years). Ninety-four (64%) of the patients were male, varying from 52% for *Kingella* spp to 78% for *Cardiobacterium* spp. The higher risk for

Table 1
Characteristics of 149 HACEK blood culture isolates.

Microorganism	Number of cases (%)	Incidence ^a	Median age in years (range)	% male
<i>Haemophilus spp</i>	55 (37)	0.16	55 (0.0–97)	64
<i>H. parainfluenzae</i>	48 (32)	0.14	54 (2–90)	65
<i>H. parahaemolyticus</i>	4 (3)	0.01	47 (0.0–69)	100
<i>Haemophilus spp</i> not specified	3 (2)		72 (0.1–97)	0
<i>Aggregatibacter spp</i>	37 (25)	0.11	69 (18–96)	73
<i>A. actinomycetemcomitans</i>	18 (12)	0.05	71 (27–96)	72
<i>A. aphrophilus</i>	12 (8)	0.04	68 (18–86)	67
<i>A. segnis</i>	6 (4)	0.02	48 (24–63)	83
<i>Aggregatibacter spp</i> not specified	1 (1)		69	100
<i>Cardiobacterium spp</i>	9 (6)	0.03	62 (24–79)	78
<i>C. hominis</i>	8 (5)	0.02	59 (24–79)	75
<i>Cardiobacterium spp</i> not specified	1 (1)		72	100
<i>Eikenella spp</i>	21 (14)	0.06	62 (2–89)	57
<i>E. corrodens</i>	20 (13)	0.06	61 (2–89)	60
<i>Eikenella spp</i> not specified	1 (1)		69	0
<i>Kingella spp</i>	27 (18)	0.08	1 (0.5–68)	52
<i>K. kingae</i>	26 (17)	0.08	1 (0.5–68)	50
<i>K. denitrificans</i>	1 (1)	0.003	1	100
Total	149	0.44	56 (0.0–97)	64

^a Per 100 000 person-years.

males was assessed by the incidence rate ratio (IRR 1.8, 95% confidence interval 1.3–2.6).

The highest age-specific incidence was found among infants, and there was a markedly increasing incidence from children to the older population, as shown in Figure 1. However, the age-specific incidences varied greatly among genera.

The overall median age was 56 years (range 0–97 years). The age distribution differed among genera, as shown in Figure 2, with the lowest median age for *Kingella spp* and the highest median age for *Aggregatibacter spp*. For *Kingella spp*, 74% of the patients were infants, while *Aggregatibacter spp* and *Cardiobacterium spp* were found mostly among patients aged 40 years and older (92% vs. 89%, respectively). *Haemophilus spp* was more evenly distributed among age groups, with patients under 40 years of age accounting for 29% of the cases. A table of the incidences according to age group, sex, and bacterial genus is found in the Supplementary material.

Discussion

In this nationwide population-based study, *Haemophilus spp* other than *H. influenzae* accounted for most cases of HACEK bacteraemia in Denmark, and *H. parainfluenzae* was the species most frequently identified. A markedly increasing incidence of HACEK bacteraemia with increasing patient age was found;

however this varied among genera. There was a notable male preponderance in HACEK bacteraemia.

It appears that no previous study has reported the incidence of HACEK bacteraemia and thus direct comparisons cannot be made. Furthermore, previous HACEK studies have focused mainly on IE rather than bacteraemia, except one study from New Zealand including 87 HACEK bacteraemia cases between 1979 and 2010 (Yew et al., 2014). The findings of that previous study differ from those of the present study regarding both the genus distribution and median age of the patients. *Aggregatibacter* was the most prevalent genus according to current taxonomy (40% of the cases), followed by *Kingella* and *Haemophilus* (24% and 23%, respectively). The median age of the New Zealand patients was lower, except for patients with *Kingella spp* and *E. corrodens*. Thus, the median age was 45 years compared to 56 years in the present Danish study. No double infections were reported, but three cases of recurrent *A. actinomycetemcomitans* bacteraemia were described among the New Zealand cases. The sex distribution in the New Zealand study, with 69% male, is similar to that in the present study.

Denmark and New Zealand are high-income industrialized countries with publicly funded health care. Still, there are both geographical and demographic differences, although the different study periods (1979–2010 vs. 2010–2015) should also be taken into account. The increase in life span has resulted in a larger group of older patients, which may render the study populations less comparable. Another explanation for the differences might be data

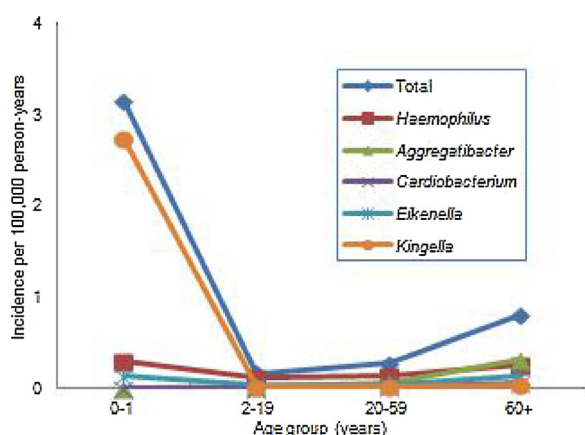


Figure 1. Age and genus-specific incidence of HACEK bacteraemia in Denmark, 2010–2015.

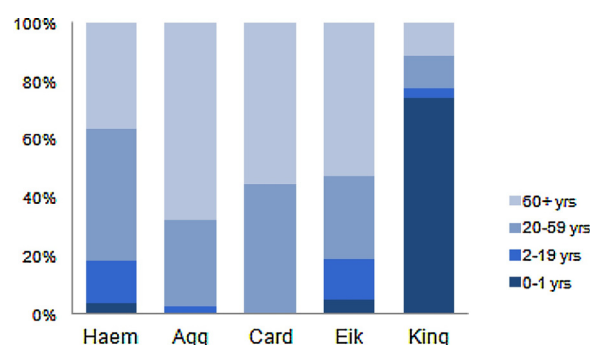


Figure 2. Age distribution for 149 blood culture isolates obtained from 147 incident cases of HACEK bacteraemia, according to bacterial genus: *Haemophilus* (Haem), *Aggregatibacter* (Agg), *Cardiobacterium* (Card), *Eikenella* (Eik), and *Kingella* (King).

completeness. The present Danish data include all incident cases of HACEK bacteraemia in the population during the study period, and little variation was seen between the five autonomous health regions, which speaks for uniformity in the diagnosis of HACEK bacteraemia. In comparison, the New Zealand data were limited to five departments of clinical microbiology with an uneven time distribution of cases, which could have led to selection and/or information bias. Furthermore, the possibility of improved bacterial identification could have contributed to the differences. The renaming of certain *Haemophilus* species as *Aggregatibacter* occurred recently in 2006 (Nørskov-Lauritsen and Kilian, 2006), and both classical phenotypic identification as well as early versions of the MALDI-TOF MS databases had difficulties in species-level identification of some of the species (Couturier et al., 2011; Haag et al., 1998; Powell et al., 2013). Current versions of Bruker Biotyper and Vitek MS databases have improved discriminatory power (Branda et al., 2014; Bruin et al., 2014; Powell et al., 2013).

The major strengths of this study are the nationwide, population-based design and the use of a national database, the equal access to public health services, and the predominant use of MALDI-TOF MS for bacterial identification, which reduced the risk of selection and information bias. However there are still some limitations to consider: the design is retrospective, data refer to the bacterial species identification reported to MiBa with no subsequent confirmation of the reported species, and in some cases phenotypic identification methods were used at the commencement of the study period because some departments had not yet implemented MALDI-TOF MS. Overall, the level of identification is considered to be good, particularly at the genus level. Clinical and treatment data were not included, which may have been helpful in explaining some of the observed differences in age, sex, and genus-specific incidence rates. Finally, this study was restricted to a high-income industrialized country, which may limit generalization to other regions.

These Danish data comprise the largest material regarding HACEK bacteraemia published so far and the first population-based study of HACEK bacteraemia incidence. HACEK bacteraemia is likely to become more prevalent in the future due to the continuing rise in life expectancy. Reliable identification by MALDI-TOF MS and nucleic acid techniques will also contribute to better ascertainment. Further studies are warranted, and are ongoing, to elucidate the association of HACEK bacteraemia with particular foci of infection in a population-based design and the clinical significance of timely detection and proper, targeted antimicrobial therapy.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical statement

The study was conducted in accordance with the regional guidelines for the use of clinical and laboratory data and was approved by the Danish Data Protection Agency (record number 2012-58-0004) and the National Board of Health (record number 3-3013-1170/1). According to Danish law, approval from a science ethics committee is not required for a register-based study.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

We would like to thank all of the Danish departments of clinical microbiology joining the MiBa collaboration for contributing their data. We would also like to thank Jonas Boel, Copenhagen University Hospital, Herlev and Gentofte Hospital, for technical support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.ijid.2018.01.025>.

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